

Fig. 1. Scheme for manufacturing microchips by method of photo-initiated polymerization. The compositions from plate (1) for micro titration is transferred by robot (2) onto a modified preparative glass (3) as droplets. The polymerization is initiated by an exposure of UV-radiation with wavelength of 312 nm. After the polymerization, biochips are washed from non-reacted ingredients, dried, and used in studies of various types.

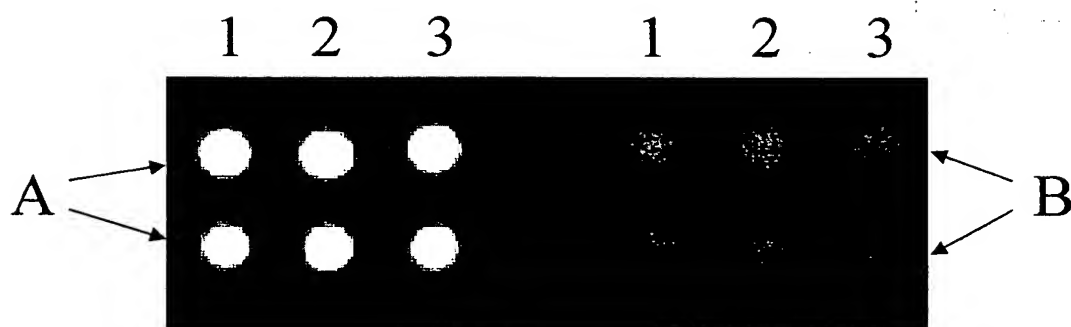
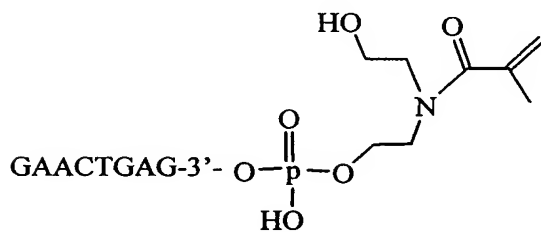
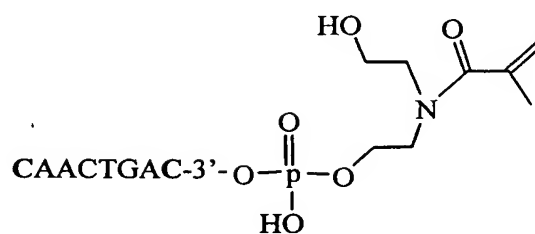


Fig. 2 Results of hybridization of the fluorescently labelled oligonucleotide over a biochip with immobilized oligonucleotides

Oligonucleotides



A



B

prepared under standard conditions of automatic synthesis with using a methacrylamide-CPG are immobilized in a gel forming part of compositions No. 1 - 3, and hybridized with the fluorescently labelled oligonucleotide Texas Red-3'-CTTGACTC.

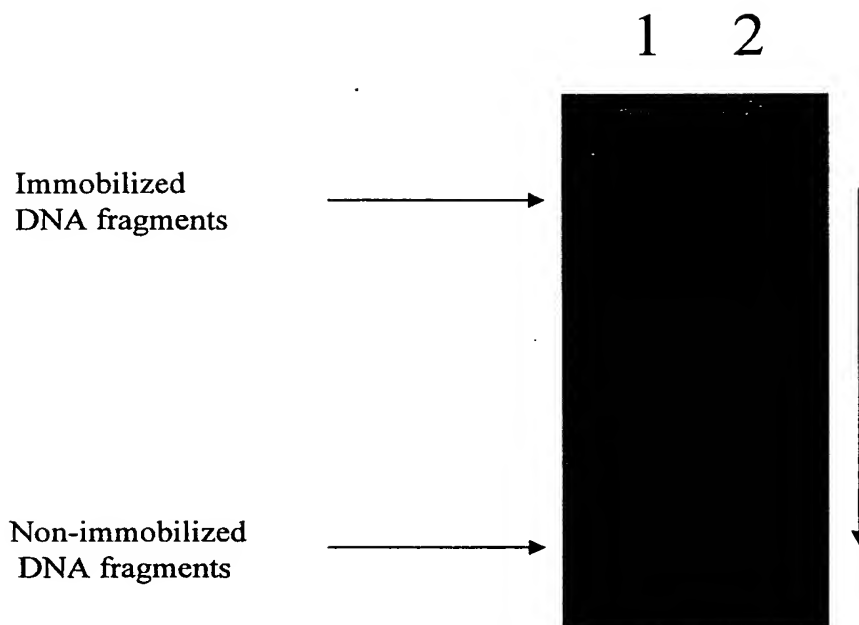


Fig. 3. Results of electrophoretic analysis of DNA

Human gene fragment ABL (334 base pairs) acylated with methacrylic acid anhydride (1) (Example 3), and the similar non-acylated DNA fragment (2) are block-copolymerized as a part of composition No. 9. For removal of the non-immobilized DNA from blocks, an electrophoresis is performed. A fluorescent picture of electrophoresis is obtained on irradiation (254 nm) of the fluorescent substrate having a polyacrylamide gel.

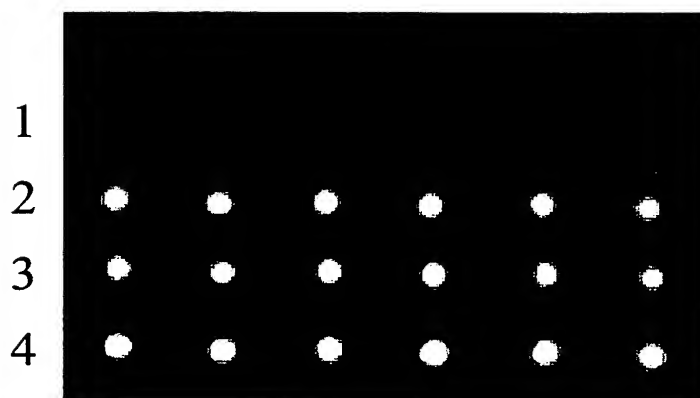


Fig. 4. Results of hybridization of fluorescent labelled probe over the biochip with the immobilized DNA fragments (human gene fragment ABL, 334 base pairs).

DNA fragments:

1. double-stranded DNA fragment prepared by PCR using the unmodified primers;
2. double-stranded DNA fragment prepared by PCR using the primer containing a 5'- methacrylamide group;
3. double-stranded DNA fragment prepared by acylation of DNA with methacrylic acid anhydride following the PCR with the unmodified primers;
4. double-stranded DNA fragment prepared by acylation of aminated DNA being obtained after the PCR with the unmodified primers, are immobilized in a gel as a part of composition No. 9 and hybridized with the fluorescently labeled oligonucleotide FITC-GTACCAGGAGTGTTTCTCCAGACTG.

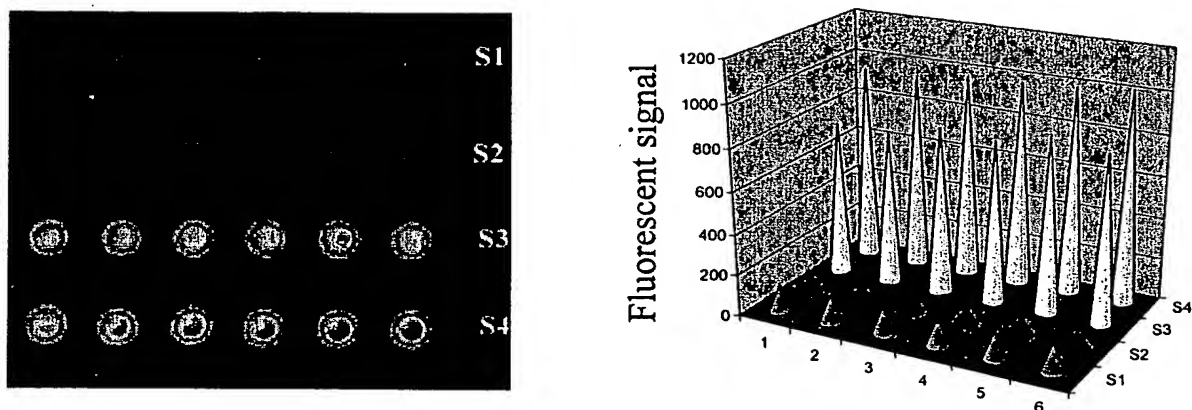


Fig. 5. The dependence of activity of protein immobilized in a hydrogel via modified amino-groups on the extent of protein modification

The modified Barnase protein having from 1 to 9 unsaturated groups inserted per one protein molecule is immobilized by method of photo-initiated copolymerization under an exposure of UV-radiation ($\lambda = 312 \text{ nm}$) over a glass modified with Bind Silane. For immobilizing, use is made of No. 4 composition (see Table 1).

A binding with a fluorescently labeled Barstar inhibitor is performed in a brine (0.1M NaCl) phosphate buffer (0.01M, pH 7.4) comprising 0.1% Tween 20, for 8 h, at 5°C; Barstar concentration is of 0.1 mg/mL.

The effect of number of groups inserted on the activity of immobilized protein is evaluated by a signal strength obtained in a fluorescent microscope.

S1 – protein free gel

S2 – totally modified protein (9 groups)

S3 – 5 groups

S4 – 1 group.

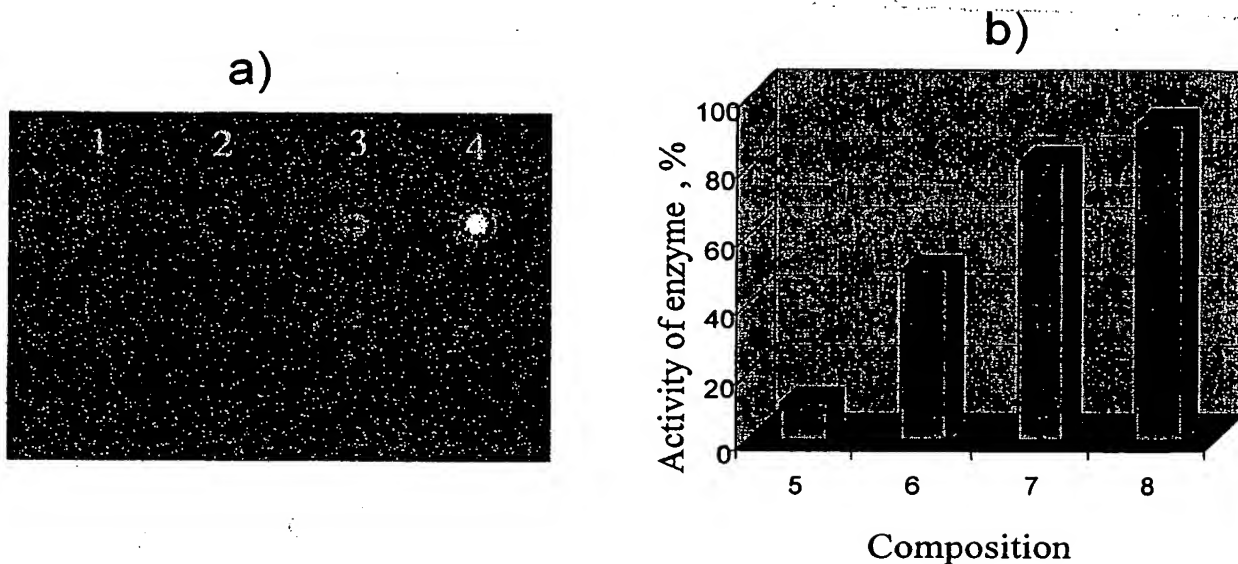


Fig. 6. Enzyme (Horseradish peroxidase) immobilization in a gel on application of compositions with various contents of sucrose and glycerol.

The modified enzyme bearing the unsaturated groups in its structure is immobilized in a gel by method of photo-initiated copolymerization over a glass modified with Bind Silane. For immobilizing, uses are made of No. 5-8 compositions (see Table 1) with various contents of sucrose and glycerol. The activity of enzyme immobilized is determined using a fluorescent microscope in performing a chemiluminescent reaction of luminol oxidation with hydrogen peroxide.

a) Biochip luminescence after performing an enzymatic oxidation:

1, 2, 3, 4 are No. 5-8 compositions respectively

b) Relative activity of enzyme after immobilization: 1- activity of enzyme, %; 2- composition numbers.

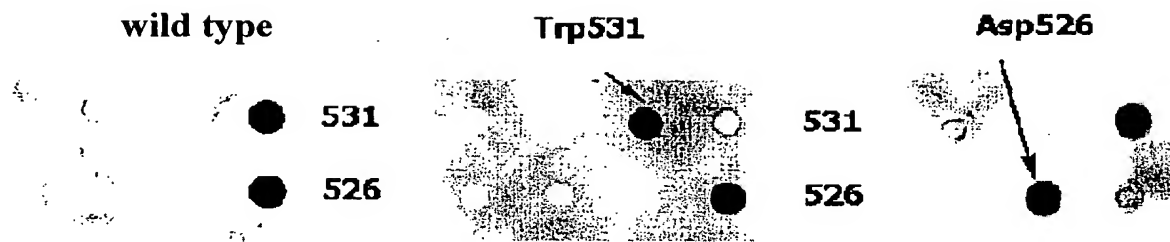


Fig. 7. Detection of mutations in 526 and 531 gene codons coding RNA-polymerase of *M. tuberculosis* by using the PCR over a biochip obtained by a copolymerization method.

There are performed three independent experiments using the genomic DNA of *M. tuberculosis* of wild type or DNA comprising a known mutation (Trp531 or Asp526) as the objects of study.

Scheme of the chip

1	2	3	4	
○	○	○	○	531
○	○	○	○	526

Structure of oligonucleotide-primers immobilized over a biochip by a copolymerization method:

531 codon

- 1.leu531 GGTTGACCCACAAGCGCCGACTGTT
- 2.cys531 GGTTGACCCAtAAGCGCCGACTGTGT
- 3.trp531 GGTTGACCACAAGCGCCGACTGTGG
- 4.ser531 GGTTGACCCACAAGCGCCGACTGTC (wild type)

526 codon

- 1.Asn526 CCAGAACAACaCGCTGTCGGGGTTGACCA
- 2.Tyr526 CCAGAACAACaCGCTGTCGGGGTTGACCT
- 3.Asp526 CCAGAACAACCCGCTGTCGtGGTTGACCG
- 4.His 526 CCAGAACAACCCGCTGTCGGtGTTGACCC (wild type)

Structure of oligonucleotide-primers used in a solution:

- F (direct): 5'-NH₂-GGTCGCCGCGATCAAGGAGT-3'
- R (reverse): 5'-NH₂-CGGCACGCTCACGTGACAGA-3'

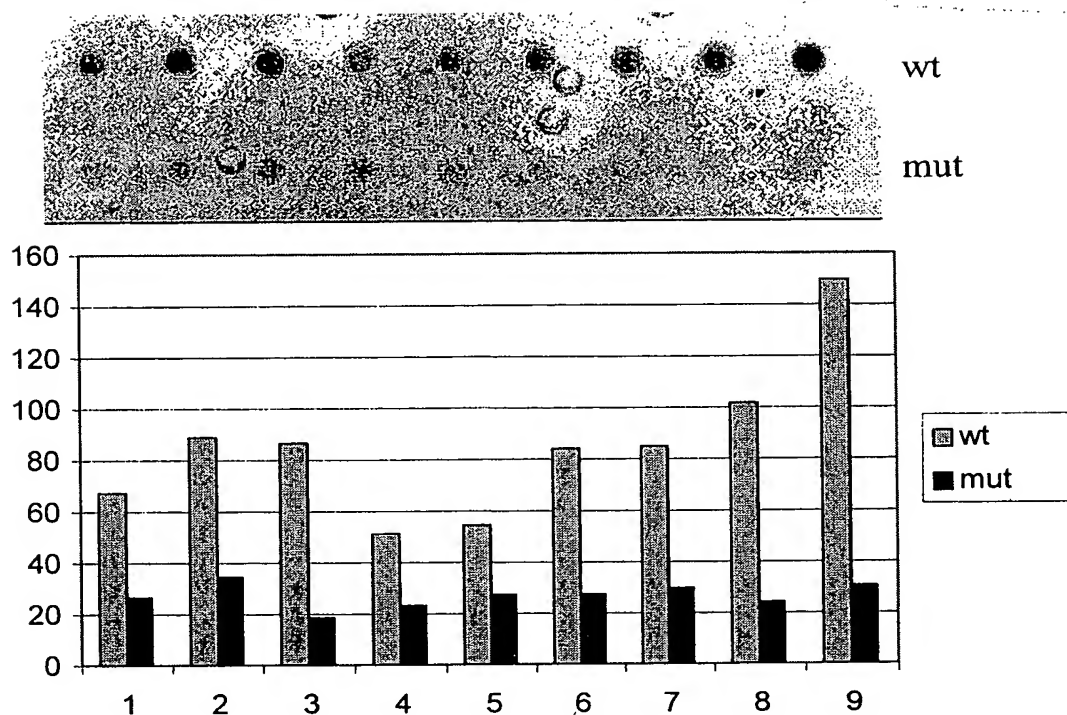


Fig. 8. Illustration of allele specific elongation of immobilized primers as a result of PCR performed inside of biochip's gel cells under mineral oil

As an object of study, use is made of genomic DNA of *M. tuberculosis* (culture of wild type).

A lower part of this figure represents the relative quantitative data on the fluorescent intensity (as a result of hybridization of the fluorescently labeled PCR-chain with the immobilized primer elongated due to PCR) in cells with the primer being entirely complementary to the DNA studied (wt) and in cells with the mutant primer (mut).

Structure of oligonucleotides immobilized over the biochip by method of copolymerization:

C4: CCAGAACAACCCGCTGTCGGtGTTGACCC (wild type)

C5: CCAGAACAACaCGCTGTCGGGGTTGACCT (Tyr:526)